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# *Escherichia coli*, *Klebsiella pneumoniae* 및 *Proteus mirabilis*에서 플라스미드매개성 AmpC $\beta$ -lactamase를 검출하는 3가지 표현형 검사법의 비교

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## Comparison of 3 Phenotypic-detection Methods for Identifying Plasmid-mediated AmpC $\beta$ -lactamase-producing *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* Strains

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**Background :** Plasmid-mediated AmpC  $\beta$ -lactamases (PABLs) have been detected in the strains of *Escherichia coli*, *Klebsiella* spp., *Proteus mirabilis*, and *Salmonella* spp. PABLs may be difficult to detect and might interfere in the therapeutic and infection-control processes. Although several PABL-detection methods based on phenotypes have been reported, the Clinical and Laboratory Standards Institute currently does not recommend a routine detection method for PABLs. The aim of this study is to compare the performances of 3 phenotypic PABL detection methods.

**Methods :** Total 276 non-duplicated clinical isolates of *E. coli* (N=97), *K. pneumoniae* (N=136), and *P. mirabilis* (N=43) were collected from 14 hospitals in Korea between April and June 2007 in a non-consecutive and non-random manner. Multiplex PCR was performed to detect the PABL genes. Further, 3 phenotypic detection methods-cephamycin-Hodge test, Tris-EDTA (TE) disk test, and combination-disk test with 3-aminophenylboronic acid (BA)-were performed using cefoxitin and cefotetan disks.

**Results :** PABL genes were detected by multiplex PCR in 122/276 isolates, including 14/97 *E. coli*, 105/136 *K. pneumoniae*, and 3/43 *P. mirabilis* isolates. The combination-disk test with BA showed higher sensitivity (98.4%), specificity (92.2%), and efficiency (96.3%) than the cephamycin-Hodge (76.2%, 96.1%, and 88.6%, respectively) and the TE-disk (80.3%, 91.6%, and 87.9%, respectively) tests.

**Conclusions :** The combination-disk test with BA is a simple, efficient, and interpretable test that can be applicable in clinical laboratories involved in the detection of PABLs in clinical isolates of *E. coli*, *K. pneumoniae*, and *P. mirabilis*. (Korean J Lab Med 2009;29:448-54)

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## INTRODUCTION

AmpC  $\beta$ -lactamases hydrolyze extended-spectrum cephalosporins and are present in many gram-negative bacilli. *ampC* genes are originally chromosomal in *Citrobacter* spp., *Enterobacter* spp., *Morganella* spp., *Hafnia* spp., and *Serratia* spp. [1]. Since 1989, plasmid-mediated AmpC  $\beta$ -lactamases (PABLs) have been detected worldwide in the strains of *Escherichia coli*, *Klebsiella* spp., *Proteus mirabilis*, and *Salmonella* spp. [1, 2] Decreased susceptibility to cefoxitin in *Enterobacteriaceae* may indicate the involvement of AmpC, but cefoxitin resistance may also be attributable to the decrease in the outer membrane permeability [3]. PABL-producing strains may also be susceptible to cefoxitin and cefotetan according to the current breakpoint criteria [4]. It may be difficult to detect PABLs, which are known to interfere with the therapeutic and infection control processes [5]. Therefore, it is important to develop methods that can easily and appropriately detect PABLs in clinical isolates. Although several phenotypic methods for their detection have been reported, CLSI currently does not recommend testing for PABLs [6]. Phenotypic-detection methods can be divided into following 2 categories: ones that detect AmpC activities in enzyme extracts and the others that evaluate the inhibitory effects induced by AmpC inhibitors. Three-dimensional extraction test has been reported as a reliable enzyme-extraction method, but this method is complicated and not applicable to clinical microbiology laboratories [7]. Cephamycin-Hodge test and Tris-EDTA (TE)-disk test were reported as simple and sensitive phenotypic-detection methods [5, 8]. Boronic acid (BA), cloxacillin, and Syn2190 have been identified as AmpC inhibitors [7, 9, 10]. Several phenotypic-detection methods have been developed using these inhibitors, but the one developed using BA, which was first introduced by Yagi et al. [11], is relatively available. The aim of this study is to compare the performance of the following 3 phenotypic-detection methods: cephamycin-Hodge test, TE-disk test, and combination-disk test with BA, for identifying PABL-producing *E. coli*, *K. pneumoniae*, and *P. mirabilis* strains.

## MATERIALS AND METHODS

### 1. Strains and antibiotic susceptibility test

A total of 276 non-duplicated clinical isolates of *E. coli* (N=97), *K. pneumoniae* (N=136), and *P. mirabilis* (N=43) were collected from 14 hospitals in Korea between April and June 2007 in a non-consecutive and non-random manner. Disk-diffusion-susceptibility tests for cefoxitin, cefotetan, and cefepime were performed according to the CLSI recommendations [12].

### 2. Multiplex PCR for plasmid-mediated *ampC* genes

All the isolates were tested for plasmid-mediated *ampC* genes using multiplex PCR developed by Perez-Perez and Hanson [13].

### 3. Cephamycin-Hodge test

The surfaces of MacConkey agar (MCA) plates were inoculated with a lawn of the indicator strain, *E. coli* ATCC 25922, according to the CLSI disk-diffusion method [12]. After the agar surface dried, a test strain was heavily streaked from the center of the plate to the periphery and a cephamycin disk (BD Diagnostic Systems, Sparks, MD, USA) was placed at the center. The plates were incubated overnight at 35°C, and the presence of definite growth of the indicator organism in the inhibition zone along with the test strain was interpreted as positive.

### 4. TE-disk test

TE disks were prepared in-house by applying 20  $\mu$ L of a 1:1 mixture of saline and 100 $\times$  Tris-EDTA (Sigma-Aldrich Corp., St. Louis, MO, USA) to sterile filter paper disks, following which the disks were dried and stored at 2–8°C. A lawn of *E. coli* ATCC 25922 was inoculated on the surface of a Mueller-Hinton agar (MHA) plate. Immediately prior to incubation, a TE disk premoistened with 20  $\mu$ L of saline was inoculated with several colonies of the test organism

and placed face down on the MHA plate adjacent to a disk containing any one of the following: cephamycins, ceftiofur, or cefotetan. The plates were incubated overnight, and an indentation in the zone of inhibition was interpreted as positive [5].

### 5. Combination-disk test with boronic acid

3-Aminophenylboronic acid (120 mg; Sigma-Aldrich Corp., St. Louis, MO, USA) was dissolved in 3 mL of dimethyl sulfoxide and 3 mL of sterile distilled water was added to this solution [4]. Next, 20  $\mu$ L of the BA solution was dispensed onto disks containing ceftiofur or cefotetan. A test strain was inoculated on MHA plates according to the CLSI guideline [12]. Disks containing ceftiofur, cefotetan, ceftiofur plus 400  $\mu$ g of BA, and cefotetan plus 400  $\mu$ g of BA were plated on the MHA plates. The plates were incubated overnight, and an increase in the zone size of  $\geq 5$  mm for either ceftiofur or cefotetan in the presence of BA compared with that of either drug alone was considered as positive result [4, 11].

### 6. Data analysis

Sensitivity was defined as the probability that a positive test result (TP) indicates the presence of PABL on the basis of the plasmid-mediated *ampC* gene-producing strains (PCR+), i.e., TP/PCR+. Specificity was defined as the prob-

ability that a negative test result (TN) excludes the presence of PABL from the plasmid-mediated *ampC* gene non-producing strains (PCR-), i.e., TN/PCR-. Efficiency (accuracy) was defined as the ratio of the true findings (true-positive+true-negative results) to all the test results. For comparing the efficacy of the phenotypic-detection methods, we used the Cochran Q test, and a *P* value  $<0.05$  was considered statistically significant. Statistical analyses were performed using Microsoft Excel 2000 (Microsoft Corporation, Redmond, WA, USA).

## RESULTS

All the AmpC PCR-positive strains were non-susceptible to ceftiofur, while 14 strains (9 *K. pneumoniae*, 4 *E. coli*, and 1 *P. mirabilis*) were susceptible to cefotetan (Table 1). Of the 276 strains tested, 122 were positive with the multiplex PCR (*E. coli*, 14/97; *K. pneumoniae*, 105/136; and *P. mirabilis*, 3/43). DHA group genes were predominantly observed in *E. coli* (N=8) and *K. pneumoniae* (N=102) and were also observed in 1 of the 3 PABL-producing *P. mirabilis*. MOX group genes were detected in all the studied species, but the prevalence was relatively low in *E. coli* (N=2) and *K. pneumoniae* (N=1). Of the 3 PABL-producing *P. mirabilis*, 2 were MOX-type enzyme producers. CIT group genes (CMY-like genes originated from *Citrobacter freundii*) were detected in only 3 strains of *E. coli* [13]. Amplicons of EBC group

**Table 1.** Disk diffusion results of ceftiofur, cefotetan, and ceftazidime compared with results of AmpC PCR

Species	AmpC PCR	N of isolates								
		Ceftiofur			Cefotetan			Ceftazidime		
		S	I	R	S	I	R	S	I	R
<i>E. coli</i>	+			14	4	2	8	13	1	
	-	51	11	21	72	7	4	73	4	6
<i>K. pneumoniae</i>	+		1	104	9	15	81	100	2	3
	-	15	6	10	27	2	2	26	4	1
<i>P. mirabilis</i>	+		1	2	1	1	1	3		
	-	37	1	2	39		1	40		
All	+	0	2	120	14	18	90	116	3	3
	-	103	18	33	138	9	7	139	8	7
Sensitivity (%)			100.0			88.5			-	
Specificity (%)			66.9			89.6			-	

Abbreviations: S, susceptible; I, intermediate; R, resistant.

genes (including MIR-1 and ACT-1, which were originated from *Enterobacter cloacae*) were observed in 1 *E. coli* and 2 *K. pneumoniae* strains [13] (Table 2).

A total of 93 PABL-producing strains were positive with the cephamycin-Hodge test, but 29 PABL-producing strains were not detected by this method. The overall sensitivity, specificity, and efficiency of the cephamycin-Hodge test were 76.2%, 96.1%, and 88.6%, respectively. By TE-disk test, 98 strains were determined as PABL-producers, while 24 strains were false-negatives. The overall sensitivity, specificity, and efficiency of the TE-disk test were 80.3%, 91.6%, and 87.9%, respectively. Combination-disk test with BA detected 120 strains of the 122 PABL-producers, but 12 PABL-

non-producers were also tested positive by this method. The overall sensitivity, specificity, and efficiency of the combination-disk test with BA were 98.4%, 92.2%, and 96.3%, respectively (Table 3). The efficiency of the combination-disk test with BA was significantly better than that of the former 2 methods (Cochran Q=16.1,  $P<0.05$ ).

The performance of the phenotypic-detection methods was also analyzed on the basis of the tested antibiotic disks, i.e., ceftioxin or cefotetan. The sensitivity of ceftioxin-Hodge test was 74.6%, while that of cefotetan-Hodge was 14.8%. A similar result was obtained by TE-disk test when it was evaluated with a single disk of ceftioxin or cefotetan. The most sensitive results were obtained with the combination disk test with BA: the sensitivity was 86.9% with ceftioxin and 89.3% with cefotetan (Table 4).

**Table 2.** Distribution of plasmid mediated *ampC* genes within study isolates

Species	Types of <i>ampC</i> genes				
	CIT	DHA	EBC	MOX	Total
<i>E. coli</i>	3	8	1	2	14
<i>K. pneumoniae</i>	0	102	2	1	105
<i>P. mirabilis</i>	0	1	0	2	3

## DISCUSSION

There is no CLSI guidelines for phenotypic methods to screen and detect AmpC activity in clinical isolates of *E. coli*,

**Table 3.** Comparison of 3 phenotypic detection methods for plasmid-mediated AmpC  $\beta$ -lactamase producing-isolates

Species	AmpC PCR	Ceftioxin		Hodge		TE-disk	
		Positive (%)	Negative (%)	Positive (%)	Negative (%)	Positive (%)	Negative (%)
<i>E. coli</i>	Positive	13 (92.9)	1 (7.1)	5 (35.7)	9 (64.3)	9 (64.3)	5 (35.7)
	Negative	10 (12.0)	73 (88.0)	1 (1.2)	82 (98.8)	3 (3.6)	80 (96.4)
<i>K. pneumoniae</i>	Positive	104 (99.0)	1 (1.0)	86 (81.9)	19 (18.1)	88 (83.8)	17 (16.2)
	Negative	2 (6.5)	29 (93.5)	2 (6.5)	29 (93.5)	7 (22.6)	2 (77.4)
<i>P. mirabilis</i>	Positive	3 (100)	0 (0.0)	2 (66.7)	1 (33.3)	1 (33.3)	2 (66.7)
	Negative	0 (0.0)	40 (100)	3 (7.5)	37 (92.5)	3 (7.5)	37 (92.5)
All	Positive	120 (98.4)	2 (1.6)	93 (76.2)	29 (23.8)	98 (80.3)	24 (19.7)
	Negative	12 (7.8)	142 (92.2)	6 (3.9)	148 (96.1)	13 (8.4)	141 (91.6)
Efficiency		96.3%		88.6%		87.9%	

**Table 4.** Sensitivity and specificity of the plasmid-mediated AmpC  $\beta$ -lactamase detection methods depending on the antibiotics used (%)

Species		Boronic acid		Hodge		TE-disk	
		Ceftioxin	Cefotetan	Ceftioxin	Cefotetan	Ceftioxin	Cefotetan
<i>E. coli</i>	Sensitivity	78.6	71.4	28.6	21.4	64.3	21.4
	Specificity	88.0	92.8	98.8	98.8	96.4	100.0
<i>K. pneumoniae</i>	Sensitivity	87.6	92.4	81.0	13.3	83.8	1.9
	Specificity	93.5	100.0	93.5	100.0	77.4	100.0
<i>P. mirabilis</i>	Sensitivity	100.0	66.7	66.7	33.3	33.3	33.3
	Specificity	97.5	100.0	92.5	100.0	92.5	100.0
All	Sensitivity	86.9	89.3	74.6	14.8	80.3	4.9
	Specificity	91.6	96.1	96.1	99.4	91.6	100.0

*K. pneumoniae*, and *P. mirabilis* [6]; these strains could be non-susceptible to cephamycin mainly because of PABLs. Although several phenotypic screening methods have been used in clinical microbiology laboratories for the detection of PABLs in clinical isolates, only a few studies have thus far evaluated the performance of various phenotypic screening methods [14, 15]. Therefore, we attempted to compare the performance of the commonly used phenotypic screening methods.

Our data showed that all plasmid-mediated *ampC* gene producers were non-susceptible to ceftiofur, while some were susceptible to cefotetan. Therefore, ceftiofur was considered as a better PABL-screening indicator than cefotetan. This could be because of the fact that cefotetan is more active than ceftiofur [16].

In this study, 4 groups of *ampC* genes, namely, CIT, DHA, EBC, or MOX, were analyzed, and ACC- and FOX-type AmpC producers were not included in the analysis. Therefore, the performance for ACC and FOX enzyme-producing strains might be different from that of the strains analyzed in this study.

We have reported that more sensitive results can be obtained if modified-Hodge tests are conducted on MCA plates (reported in the 18th ECCMID, 2008, P-892). Lee et al. reported in the 18th ECCMID, showed that bile improved the performance of modified-Hodge test when MCA was used (2008, P-891). Although we used carbapenemase-producing strains in this study, it was apparent that better results could have been obtained by performing cephamycin-Hodge test using MCA. Therefore, we used MCA instead of MHA to detect AmpC  $\beta$ -lactamase-producing strains.

The usefulness of the antimicrobial-disk-susceptibility test using cephamycins as the phenotypic screening test for PABL-producers was investigated. Because all PABL producers were non-susceptible to ceftiofur, the susceptibility to ceftiofur can be used to rule out the possibility of PABL producers (Table 1). Several strains could be regarded as false-positives without even conducting the phenotypic AmpC confirmatory test; therefore, there might be serious risks involved in the selection of antimicrobial agents for the treatment of infections caused by these strains. The

sensitivity of cefotetan susceptibility was higher (88.5%) than Hodge test or TE disk test, but not higher than that of the combination-disk test; however, the specificity was the lowest (89.6%) compared to that of all the 3 phenotypic-detection methods (Table 1, 3). Although the possibility of obtaining false-positive results was reduced by using cefotetan, the sensitivity also decreased. The results of ceftiofur susceptibility seemed to be unrelated to PABL-producers (Table 1). Resistance to ceftiofur as well as to oxyimino- $\beta$ -lactams is not specific to AmpC enzyme because ceftiofur resistance can also be produced by certain other enzymes or by decreased permeability of the outer membrane [17–22]. Other confirmatory tests are required in this regard [23].

The results of the 3 methods varied depending on the antibiotics and bacterial species used. Hodge test and TE-disk test performed using ceftiofur showed better results than those performed using cefotetan, but the combination-disk test with BA showed relatively better results using cefotetan instead of ceftiofur (Table 4). This could be because of the increase in the inhibitory effects of BA by the higher activity of cefotetan. Using ceftiofur, the sensitivity of the combination-disk test with BA was 86.9%. Tan et al. [14] reported a 94% sensitivity of the combination-disk test with BA when the zone size of  $\geq 4$  mm was used as a positive criterion. We obtained a 92.6% sensitivity using the same cutoff criterion, but the specificity was reduced from 91.6% to 87.0%. On the other hand, when the test was performed with cefotetan alone using the same criterion, the sensitivity of the combination-disk test increased to 95.1% from 86.9% (data not shown). Collectively, the best results were obtained when each method was considered using both ceftiofur and cefotetan. The sensitivity of the combination-disk test with BA was 98.4% when the results were evaluated using both ceftiofur and cefotetan and when the increase in zone size of  $\geq 5$  mm was used as a positive criterion (Table 3). In addition, Song et al. [15] reported that the combination of ceftiofur-BA and cefotetan-BA disk tests detected 98.4% of PABL-producing strains. Two PABL-producing strains—1 *E. coli* and the other *K. pneumoniae*—showed false-negative results when combination-disk test with BA was used, and all the *ampC* gene types of these strains were of the DHA group.

Of the 12 false-positive strains, 10 were *E. coli* (Table 3). Such phenotypic results for these *E. coli* strains might be because of the increased activities of chromosomal AmpCs, but it is necessary to determine the cause of obtaining false-positive results in the 2 *K. pneumoniae* strains. The sensitivities of the Hodge test and TE-disk test were 76.2% and 80.3%, respectively. It was difficult and obscure to interpret the results of the Hodge test or TE-disk test, and the test procedures were relatively more complex than the procedure of the combination-disk test. Among the 3 methods tested, the combination-disk test was found to be the most sensitive and efficient test (Table 3). However, recently, a study showed that 3-aminophenylboronic acid inhibited the activity of KPC-type  $\beta$ -lactamase [24]. Doi et al. [24] reported that 3-aminophenylboronic acid could be used for detecting the expression of KPC-type  $\beta$ -lactamase in *K. pneumoniae* and *E. coli*. The only limitation of our study is that it lacked KPC-type  $\beta$ -lactamase-producing strains.

In conclusion, we evaluated the performance of 3 phenotypic-detection methods for identifying PABL-producing *E. coli*, *K. pneumoniae*, and *P. mirabilis* strains. The susceptibility to cefoxitin can be used as a better screening indicator than that to cefotetan. The performance of the 3 screening methods can be increased by using both cefoxitin and cefotetan. The combination-disk test with BA is a simple, sensitive, and interpretable test that can be applicable in clinical laboratories involved in the detection of PABL-producing *E. coli*, *K. pneumoniae*, and *P. mirabilis* strains.

## REFERENCES

1. Yang K and Guglielmo BJ. Diagnosis and treatment of extended-spectrum and AmpC  $\beta$ -lactamase-producing organisms. *Ann Pharmacother* 2007;41:1427-35.
2. Papanicolaou GA, Medeiros AA, Jacoby GA. Novel plasmid-mediated  $\beta$ -lactamase (MIR-1) conferring resistance to oxyimino- and alpha-methoxy  $\beta$ -lactams in clinical isolates of *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 1990;34:2200-9.
3. Ardanuy C, Linares J, Dominguez MA, Hernandez-Alles S, Benedi VJ, Martinez-Martinez L. Outer membrane profiles of clonally related *Klebsiella pneumoniae* isolates from clinical samples and activities of cephalosporins and carbapenems. *Antimicrob Agents Chemother* 1998;42:1636-40.
4. Coudron PE. Inhibitor-based methods for detection of plasmid-mediated AmpC  $\beta$ -lactamases in *Klebsiella* spp., *Escherichia coli*, and *Proteus mirabilis*. *J Clin Microbiol* 2005;43:4163-7.
5. Black JA, Moland ES, Thomson KS. AmpC disk test for detection of plasmid-mediated AmpC  $\beta$ -lactamases in *Enterobacteriaceae* lacking chromosomal AmpC  $\beta$ -lactamases. *J Clin Microbiol* 2005;43:3110-3.
6. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Seventeenth informational supplement, M100-S17. Wayne, PA: Clinical and Laboratory Standards Institute, 2007.
7. Coudron PE, Moland ES, Thomson KS. Occurrence and detection of AmpC  $\beta$ -lactamases among *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* isolates at a veterans medical center. *J Clin Microbiol* 2000;38:1791-6.
8. Yong D, Park R, Yum JH, Lee K, Choi EC, Chong Y. Further modification of the Hodge test to screen AmpC  $\beta$ -lactamase (CMY-1)-producing strains of *Escherichia coli* and *Klebsiella pneumoniae*. *J Microbiol Methods* 2002;51:407-10.
9. Netzel TC, Jindani I, Hanson N, Turner BM, Smith L, Rand KH. The AmpC inhibitor, Syn2190, can be used to reveal extended-spectrum  $\beta$ -lactamases in *Escherichia coli*. *Diagn Microbiol Infect Dis* 2007;58:345-8.
10. Beesley T, Gascoyne N, Knott-Hunziker V, Petursson S, Waley SG, Jaurin B, et al. The inhibition of class C  $\beta$ -lactamases by boronic acids. *Biochem J* 1983;209:229-33.
11. Yagi T, Wachino J, Kurokawa H, Suzuki S, Yamane K, Doi Y, et al. Practical methods using boronic acid compounds for identification of class C  $\beta$ -lactamase-producing *Klebsiella pneumoniae* and *Escherichia coli*. *J Clin Microbiol* 2005;43:2551-8.
12. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Approved Standard, M02-A9. Wayne, PA: Clinical and Laboratory Standards Institute, 2006.
13. Perez-Perez FJ and Hanson ND. Detection of plasmid-mediated AmpC  $\beta$ -lactamase genes in clinical isolates by using multiplex PCR. *J Clin Microbiol* 2002;40:2153-62.
14. Tan TY, Ng LS, He J, Koh TH, Hsu LY. Evaluation of screening methods to detect plasmid-mediated AmpC in *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*. *Antimicrob Agents Chemother*

- 2009;53:146-9.
15. Song W, Jeong SH, Kim JS, Kim HS, Shin DH, Roh KH, et al. Use of boronic acid disk methods to detect the combined expression of plasmid-mediated AmpC  $\beta$ -lactamases and extended-spectrum  $\beta$ -lactamases in clinical isolates of *Klebsiella* spp., *Salmonella* spp., and *Proteus mirabilis*. *Diagn Microbiol Infect Dis* 2007;57:315-8.
16. Ayers LW, Jones RN, Barry AL, Thornsberry C, Fuchs PC, Gavan TL, et al. Cefotetan, a new cephamycin: comparison of in vitro antimicrobial activity with other cephems,  $\beta$ -lactamase stability, and preliminary recommendations for disk diffusion testing. *Antimicrob Agents Chemother* 1982;22:859-77.
17. Wachino J, Doi Y, Yamane K, Shibata N, Yagi T, Kubota T, et al. Molecular characterization of a cephamycin-hydrolyzing and inhibitor-resistant class A  $\beta$ -lactamase, GES-4, possessing a single G170S substitution in the omega-loop. *Antimicrob Agents Chemother* 2004;48:2905-10.
18. Poirel L, Naas T, Nicolas D, Collet L, Bellais S, Cavallo JD, et al. Characterization of VIM-2, a carbapenem-hydrolyzing metallo- $\beta$ -lactamase and its plasmid- and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France. *Antimicrob Agents Chemother* 2000;44:891-7.
19. Martinez-Martinez L, Pascual A, Hernandez-Alles S, Alvarez-Diaz D, Suarez AI, Tran J, et al. Roles of  $\beta$ -lactamases and porins in activities of carbapenems and cephalosporins against *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 1999;43:1669-73.
20. Martinez-Martinez L, Hernandez-Alles S, Alberti S, Tomas JM, Benedi VJ, Jacoby GA. In vivo selection of porin-deficient mutants of *Klebsiella pneumoniae* with increased resistance to cefoxitin and expanded-spectrum-cephalosporins. *Antimicrob Agents Chemother* 1996;40:342-8.
21. Hernandez-Alles S, Conejo M, Pascual A, Tomas JM, Benedi VJ, Martinez-Martinez L. Relationship between outer membrane alterations and susceptibility to antimicrobial agents in isogenic strains of *Klebsiella pneumoniae*. *J Antimicrob Chemother* 2000;46:273-7.
22. Hernandez-Alles S, Benedi VJ, Martinez-Martinez L, Pascual A, Aguilar A, Tomas JM, et al. Development of resistance during antimicrobial therapy caused by insertion sequence interruption of porin genes. *Antimicrob Agents Chemother* 1999;43:937-9.
23. Jacoby GA. AmpC  $\beta$ -lactamases. *Clin Microbiol Rev* 2009;22:161-82.
24. Doi Y, Potoski BA, Adams-Haduch JM, Sidjabat HE, Pasculle AW, Paterson DL. Simple disk-based method for detection of *Klebsiella pneumoniae* carbapenemase-type  $\beta$ -lactamase by use of a boronic acid compound. *J Clin Microbiol* 2008;46:4083-6.